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## Measuring Glucose Levels in Tears as an Alternative to Blood Glucose levels

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# **Measuring Glucose Levels in Tears as an Alternative to Blood Glucose Levels**

Senior Project submitted to  
The Division of Science, Mathematics and Computing  
of  
Bard College

by  
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Annandale-on-Hudson, New York  
May 2016

## ***Dedication***

*This project is dedicated to my parents, Aida and Angel Gonzalez, and my brothers Angelo and Marlon Gonzalez. Since as long as I can remember all of you have been my inspiration and my driving force. Ya'll are the reasons why I want to succeed in life. I am eternally thankful for the support and unconditional love I have received. I love you with all of my heart.*

*Este proyecto es dedicado a mis padres, Aida y Angel Gonzalez, y a mis hermanos Angelo y Marlon Gonzalez. Desde que puedo recordar ustedes son mi inspiración y mi fuerza motriz. Ustedes son la razón por cual quiero ser exitosa en la vida. Estoy eternamente agradecida por el apoyo y el amor incondicional que he recibido. Gracias por siempre decirme que puedo hacer todo en la vida. Los amo con todo mi corazón.*

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## ***Abstract***

A person with type I and II diabetes is required to regulate and monitor the levels of glucose in their blood; this process can be painful because it requires the person to get pricked by a lancet multiple times a day. Therefore, the development of a non-invasive and pain free technique can be beneficial for patients with diabetes. We made tear and blood glucose measurements to verify the correlation between them. We used commercially available glucometers and tested other home-built alternatives glucometers with varying sensitivities and limits-of-detection in an attempt to measure tear glucose as a replacement for blood glucose measurements.

# ***Chapter 1: Introduction***

## ***1.1 What is diabetes mellitus (DM)?***

Diabetes mellitus (or diabetes) is a chronic group of metabolic disorders<sup>1</sup> that affects the way the body uses digested sugar for energy. Diabetes is categorized as having high or low blood sugar levels, otherwise known as hyperglycemia and hypoglycemia, respectively, over a prolonged period of time.<sup>2</sup> During the digestion process, the body breaks down carbohydrates into a simple sugar called glucose.<sup>3</sup> When glucose enters the bloodstream, the increase in sugar concentration signals the pancreas to produce insulin. Insulin is a hormone that helps regulate the uptake of glucose into most cells in the body. Insulin also helps the body adsorb glucose, which is then converted into energy needed by the muscles and tissues to function properly. A person with diabetes does not absorb glucose adequately, thus when glucose circulates in the blood, it can cause damage to tissues overtime. Diabetes can ultimately lead to detrimental damages in blood vessels, the eyes, kidneys, and nerves. It may also increase the risk of cardiovascular disease and lower limb amputations.<sup>4</sup> People with this disease are also at higher risks of developing infections, such as respiratory infections, urinary tract infections, skin, and soft tissue infections. Potential causes of diabetes include the pancreas not producing enough insulin and/or the cells in the body not responding appropriately to the insulin being produced. Also, physiology effects, such as obesity and physical inactivity are strongly associated with the development of type 2 diabetes.<sup>5</sup>

### ***1.1.1 Types of diabetes***

There are three main types of diabetes: type 1, type 2, and gestational diabetes<sup>4</sup> and there are other types of diabetes due to other sources, such as monogenic diabetes syndrome, diseases of the exocrine pancreas, and drug or chemical induced diabetes.<sup>6</sup> Type 1 diabetes is caused by an autoimmune response where the body's defense system attacks the insulin producing cells in the pancreas. This type of diabetes results from a cellular-mediated autoimmune destruction that is often mediated by T-cells, of the insulin-secreting cells of pancreatic  $\beta$ -cells.<sup>1,7</sup> Consequently, the pancreas fails to produce the insulin the body needs to regulate glucose and so there is an absolute deficiency of insulin.<sup>8</sup> Unfortunately, the reasons behind this mechanism are still unknown. People with type 1 diabetes may not survive without a source of daily insulin for glucose regulation.<sup>4</sup> Susceptibility to type 1 diabetes is inherited; however, the mode of inheritance is complex and has not been fully defined yet.<sup>1</sup> Additionally, environmental factors have been proven to play a role in the pathogenesis of type 1 diabetes, but this is being studied further.<sup>1</sup>

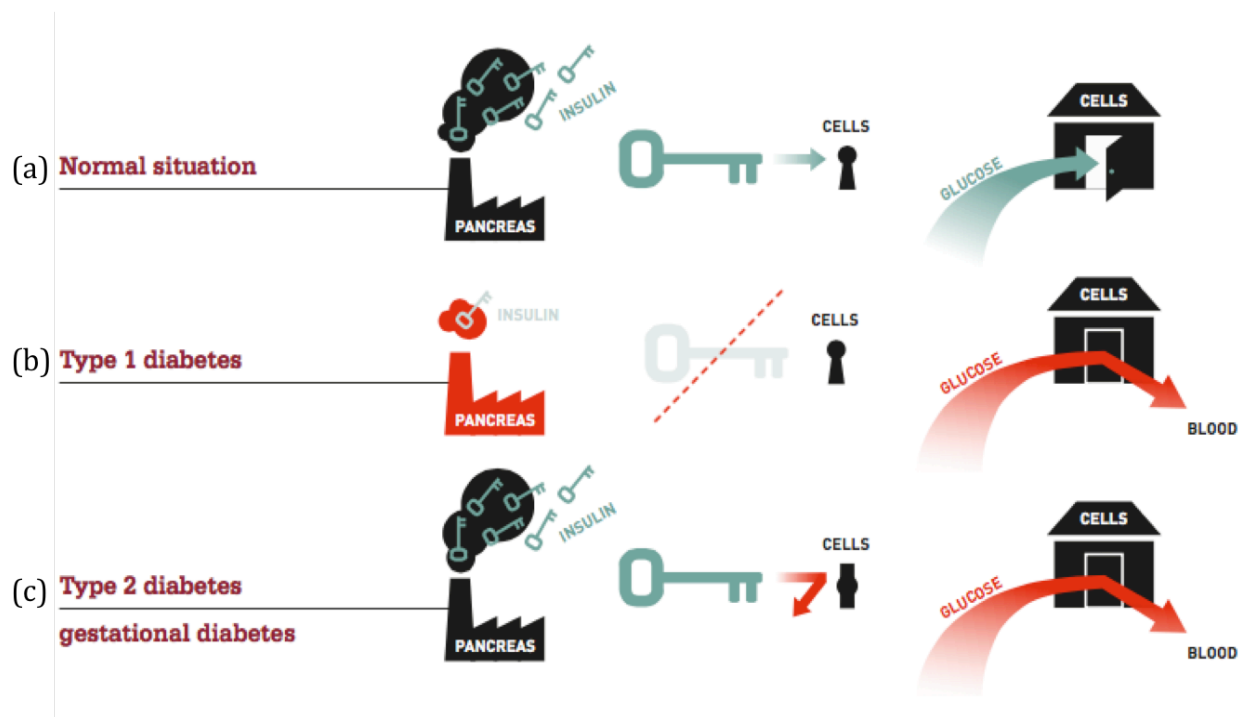
Type 2 diabetes, the most common type is caused by a combination of bodily malfunctions, namely, insulin resistance and  $\beta$ -cell dysfunction. These are pathological defects in patients with type 2 diabetes.<sup>1</sup> Insulin resistance is a decrease in the ability of insulin to act on the peripheral tissue. The  $\beta$ -cell dysfunction is the inability of the pancreas to generate the necessary insulin to compensate for insulin resistance. In other words, type 2 diabetes develops when the cells fail to respond to insulin properly or the insulin amount is not sufficient. This type of diabetes can arise from genetic or lifestyle factors. Gao *et al.* reported that type 2 diabetes is mainly caused by a decrease in peripheral tissue sensitivity to insulin as well as decreased insulin secretion from the  $\beta$ -cells. As a result of the decline



in sensitivity, the  $\beta$ -cells start to increase insulin secretion. When the  $\beta$ -cells start to overcompensate in this manner, it leads to overstraining on the  $\beta$ -cells. Overworked  $\beta$ -cells start to decrease their function, which leads to a subsequent decrease intolerance, which impairs the secretion of glucagon. Glucagon is a hormone secreted by the  $\alpha$ -cells of the pancreas that activates gluconeogenesis, which prevents hypoglycemia when no food is being digested. When glucagon is unable to achieve its function, the glycemic state of a patient worsens and leads to an accumulation of diabetic pathology.<sup>7</sup> As a result of these events, glucose accumulates in the blood at high concentrations. Type 2 diabetes may be considered the “silent killer” since many people with this condition may remain unaware of it because its symptoms may take years to develop, surface, and even be recognized. Unlike people with type 1 diabetes, type 2 diabetes patients do not require a daily dosage of insulin; instead their condition can be managed by daily physical activity, physical medication, and a healthy diet.

Gestational diabetes mellitus is defined as any degree of glucose intolerance that only develops and is recognized during the second or third trimester of pregnancy.<sup>9</sup> Normal pregnancy is associated with an increase in insulin resistance, a normal glycemic level is maintained by increased insulin secretion. Gestational diabetes develops in women who fail to increase insulin sufficiently.<sup>1</sup> This type of diabetes predisposes women and the child to an increased risk of developing type 2 diabetes later in life. Moreover, it has been linked to adverse health effects including macrosomia, shoulder dystocia, and fetal death.<sup>10</sup> Because of the health risks gestational diabetes bestows on both the mother and the child, a non-invasive, virtually pain-free technology to measure glucose would greatly benefit this group.

Figure 1 depicts three possible scenarios for the regulation of glucose. In a normal situation (Figure 1 a), the pancreas creates insulin, which promotes the absorption of glucose into cells to either store or use the blood glucose gained from food. In a person with type 1 diabetes (Figure 1 b), the pancreas is unable to produce insulin, thus the glucose cannot enter the cells, which results in glucose build up in the blood. In a person with type 2 and gestational diabetes (Figure 1 c), the body is able to make insulin, but over time the pancreas will incrementally produce less of it.<sup>11</sup> In this case, the fat, liver, and muscle cells are unable to respond correctly to insulin and therefore, glucose does not get into the cells to be stored or used for energy.



**Figure 1** Glucose pathway in non-diabetics and diabetics (Adapted from IDF Diabetes Atlas 6<sup>th</sup> edition, 2013)

### **1.1.2 How is diabetes screened and diagnosed?**

Diabetes is screened and diagnosed based on a variety of tests, such as the A1C, plasma glucose test, fasting plasma glucose (FPG) and the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT).<sup>1,12,13</sup> The A1C test also known as the hemoglobin A1C reflects a person's average blood glucose levels for the past two to three months. Specifically, this test measures the percentage of hemoglobin covered with sugar; the higher the percentage, the greater is the person's risk of diabetes complications.<sup>14</sup> An A1C level of 6.5% or higher indicates that the person has diabetes. Besides the A1C, the FPG test measures the blood glucose levels when the person has fasted for at least eight hours. A blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes, a level of 126 mg/dL (7.0 mmol/L) or higher may indicate diabetes. The 2-h PG test is similar to the FPG in that the person has to fast for at least 8 hours, but in addition, the person has to drink a 75-grams glucose drink and after two hours, another blood sample is drawn. A blood sugar level less than 140 mg/dL (7.8 mmol/L) is normal, from 140 to 199 mg/dL (7.8 to 11.0 mmol/L) is considered prediabetes, and levels above 200 mg/dL (11.1 mmol/L) may indicate diabetes.<sup>1,13</sup> Table 1 summarizes these different tests.

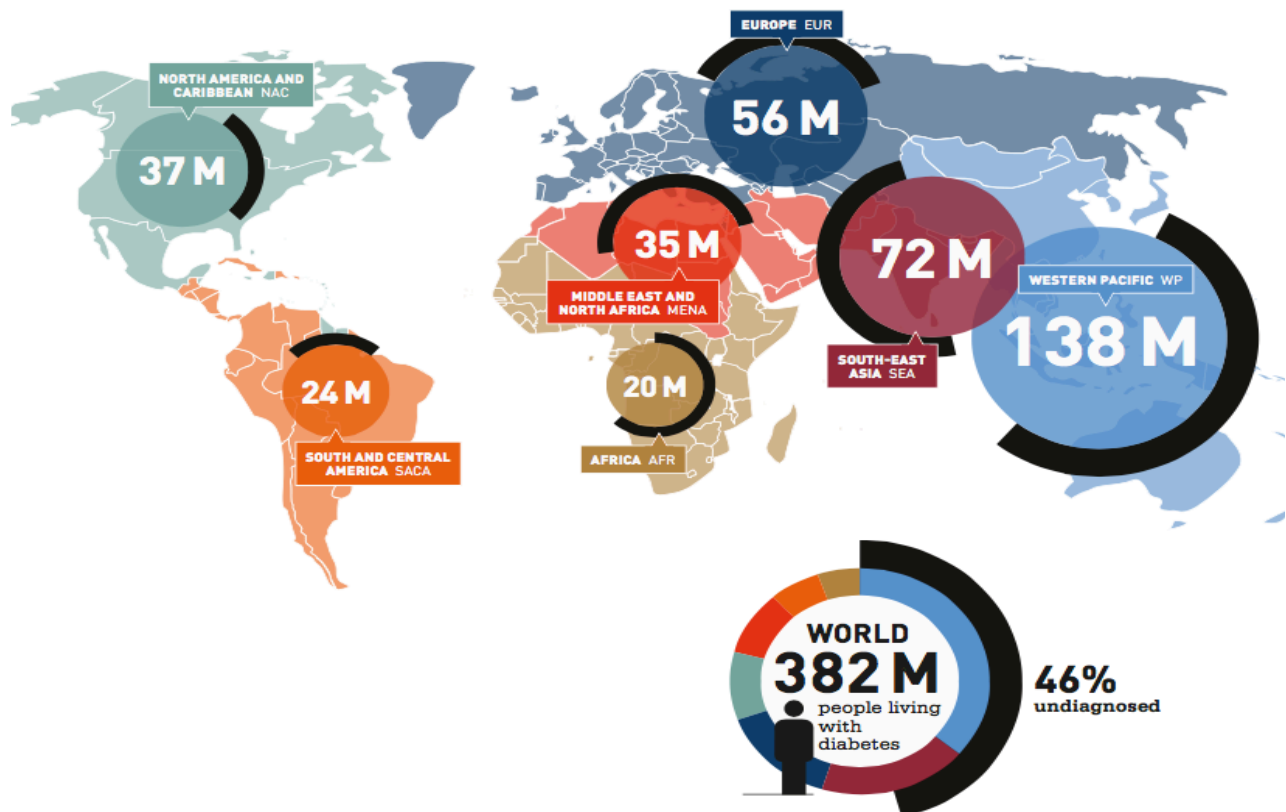
<b>Table 1: Criteria for the diagnosis of diabetes<sup>12,13</sup></b>	
<b>A1C≥6.5% (48 mmol/mol)</b>	Performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay
<b>FRP≥126 mg/dL (7.0 mmol/L)</b>	Fasting is defined as no caloric intake for at least 8 hours
<b>2-h PG≥200 mg/dL (11.1 mmol/L) during an OGTT(75-g)</b>	Performed after fasting for at least 8 hours, then test is repeated after using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water
<b>Random plasma glucose ≥200 mg/dL (11.1 mmol/L)</b>	In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis

The commonality between all these tests is that every individual with diabetes must have their blood drawn and/or prick their fingers to monitor their blood glucose levels throughout the day. These procedures are painful, discomforting, and disruptive to one's daily life. As a consequence, many people with diabetes check their blood glucose levels less often than recommended. Other complications, such as calluses after repeated pricking on fingers tips have been reported.<sup>15</sup> If a less invasive method of measuring glucose levels were offered, perhaps patient compliance would improve, allowing for a better glucose monitoring, and lower mortality rates due to complications from diabetes.

### ***1.1.3 Prevalence of Diabetes Mellitus***

Diabetes is undeniably one of the most challenging health problems in the 21<sup>st</sup> century. In recent years, 382 million people worldwide were estimated to have diabetes; by 2035 this is predicted to rise to 592 million people.<sup>4</sup> Currently, there are approximately 36.8 million people, about 9.6% of the American population, with diabetes, in addition to 86 million people who have prediabetes.<sup>4</sup> Without the proper prevention and management plans, this epidemic will continue to increase worldwide. Figure 2 shows a map with the distribution of diabetes worldwide in 2013. As depicted in the figure the Western Pacific has the most people with diabetes and Africa's population has the least people with diabetes within the regions. The majority of people with diabetes live in economically less developed regions of the world, even though Africa has the lowest prevalence, it is estimated that 522,600 people died due to diabetes in 2013.

According to the IDF Diabetes Atlas, within a country, some communities may be more vulnerable to diabetes than others because of socioeconomic disadvantage and lack of access to care. According to Gao *et al.* a survey conducted in the United States in 2008 revealed that the rates of type 2 diabetes are dependent on ethnicity as the greatest rise in childhood diabetes was largely in non-white ethnic groups. The prevalence of type 2 diabetes in European, African-American, and American-Indian communities was found to be 3, 15.7, and 49.4 out of 100,000 individuals, respectively.<sup>7</sup> Studies have shown that ethnicity may play a role in contributing to the severe impact of diabetes, but it is the social factors that form the basis for many of the chronic health problems faced by these communities.<sup>4</sup>



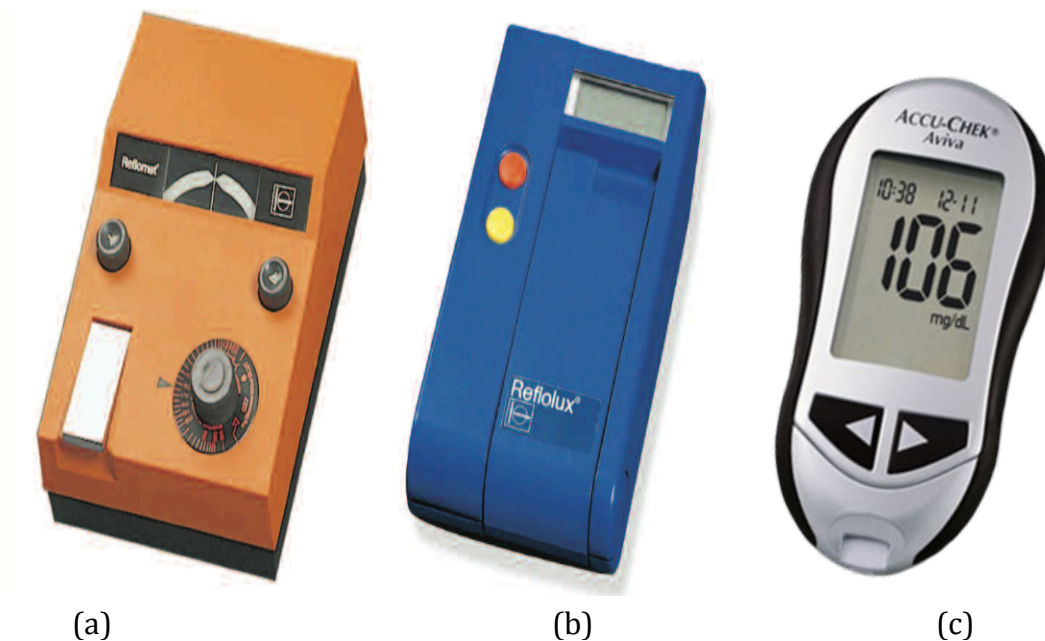
**Figure 2** Number of people with diabetes by regions around the world (adapted from IDF Diabetes Atlas 6<sup>th</sup> edition, 2013)

There is limited global prevalence data for diabetes in youth. With the increasing quantities of childhood obesity and physical inactivity, the potential for type 2 diabetes in children is rapidly increasing. The primary treatment for type 2 diabetes in children is a medication called metformin. Metformin decreases the glucose production and stimulates the glucose acceptance in peripheral tissue.<sup>7</sup> Type 1 diabetes is the most common metabolic condition in childhood. This type of diabetes requires life long insulin therapy and a management plan that consists of insulin use and blood glucose monitoring.<sup>7</sup> In many low-income families access to insulin and education about diabetes is limited; furthermore, the cost of treatment and monitoring equipment can be expensive. Consequently, children who are not able to have a proper management plan can have disabilities and even early death. According to Gao *et al.* oral insulin is not an option for these patients because of gastric enzymes that hinder oral insulin. There are many types of insulin that can be administered to children with type 1 diabetes, they all have different functioning time, but they have a similarity: the method of insulin delivery. Insulin is generally injected using a fine needle and a syringe or a pen. Many children may also use an adjustable insulin pump. A tube connects the insulin to a catheter that is inserted in the abdomen, which delivers the appropriate amount of insulin.<sup>7</sup> As of now, for patients with type 1 diabetes insulin is still being administered in an invasive manner. However, with more research, the method for measuring glucose levels may be noninvasive, in the hopes of alleviating these patients from further pain and discomfort.

## 1.2 How is glucose measured from blood?

As a way to regulate glucose levels, diabetics need to continually monitor their glucose. A blood glucose test measures the amount of glucose in the blood. Home glucometers are medical devices that allow the patient to monitor their own blood glucose concentration. Glucometers are intended to measure glucose levels in fresh capillary whole blood samples drawn from fingertips, forearm, upper arm, and palm.<sup>16</sup>

According to Tonyushkina *et al.* Ernie Adams invented the first glucometer, Dextrostix, in 1963. The Dextrostix consisted of a paper strip that develops a blue color whose intensity is proportional to glucose concentration and could be read by visually comparing the strip color to a color-concentration chart.<sup>17</sup> In 1970, Anton H. Clemens at the Ames Research Division in Indiana first developed an instrument to self-monitor blood glucose levels with Dextrostix. His invention used a dry chemistry test strip and a reflectance photometry.<sup>18</sup>



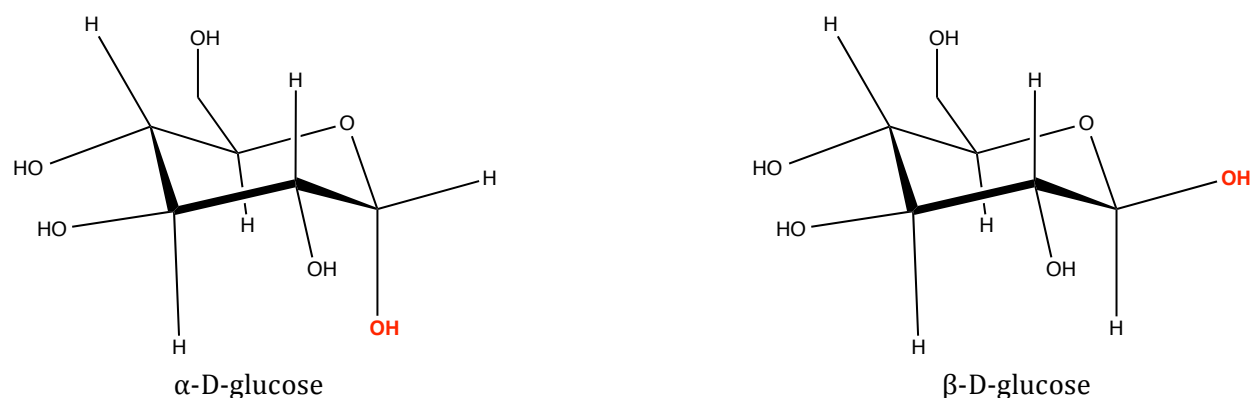
**Figure 3** Evolution of glucometers (a) Reflomat (1974), (b) Reflolux (1984)<sup>18</sup>, and the (c) Accu-Chek (2014)

In 1974, Boehringer Mannheim (BM) fabricated the Reflomat in Figure 3 (a), a reflectance meter that uses a reagent strip and small volumes of blood. Subsequently, in 1984, BM produced the Reflolux (Accu-Chek), which used improved reagent strips and small volumes of blood, as well as better visual quality.<sup>18</sup> In the 1990s these tests switched from colorimetric to electrochemical methods. A modern glucometer is one launched by Rogue Diagnostics, the Accu-Chek System, which requires 0.6 microliter volume samples yielding 5-second results and clear visual reading of the glucose concentration.<sup>19</sup>

### ***1.2.1 How do glucometers work?***

Glucose meters have two important parts: an enzymatic reaction and a detector.<sup>17</sup> Enzymes are protein catalysts, which accelerate specific chemical reactions; they are highly selective and specific, which enables the measurement of a specific type of molecule within different cells.<sup>20</sup> The enzymatic part of the glucometer is generally packaged in a dehydrated state in a disposable test strip. When blood is applied to the test strip, it rehydrates the enzyme, glucose oxidase (GOx). GOx oxidizes  $\beta$ -D-glucose almost exclusively, even in the presence of  $\alpha$ -D-glucose<sup>21</sup>, a stereoisomer (Figure 4). This reaction yields a product that can be detected either optically or electrochemically. Some meters generate hydrogen peroxide, which then can be measured electrochemically. Others have an intermediary that can react with a dye, such that the converted dye concentration is proportional to the concentration of glucose.<sup>18</sup>

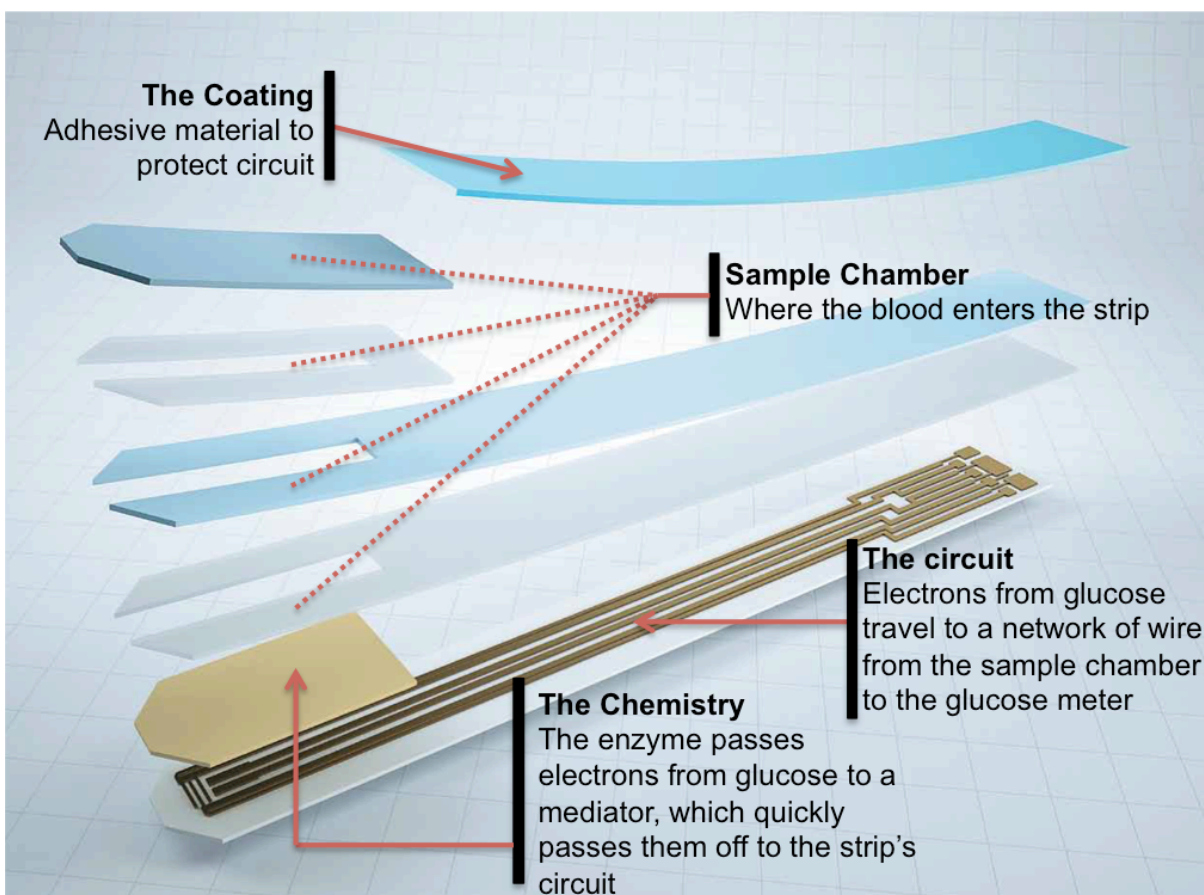




**Figure 4** Stereoisomers  $\alpha$ -D-glucose (left) and  $\beta$ -D-glucose (right)

Test strips have evolved substantially since their initial invention in the early 1980s. The first test strips measured blood glucose using an enzyme to convert the glucose in a drop of blood into a proportional amount of dye. The glucometers during this time measured the amount of dye by shining a beam of light on the test spot and detecting how much of the light was absorbed by the dye. The problem with this procedure was that it involved a lot of steps and required approximately 1-3  $\mu$ L of blood<sup>22</sup>, thus making it very time consuming and perhaps painful.<sup>23</sup>

In the early 1990s test strips changed dramatically when they began to use electrochemical detection schemes. Electrochemical strips also employ enzymes, but they convert glucose into an electrical current.<sup>23</sup> The current is then read out as being proportional to the glucose concentration. Even after this breakthrough, test strips remained imperfect; the enzyme still remains a great challenge because any alteration in its concentration or activity can decrease the accuracy of the resulting measurement.

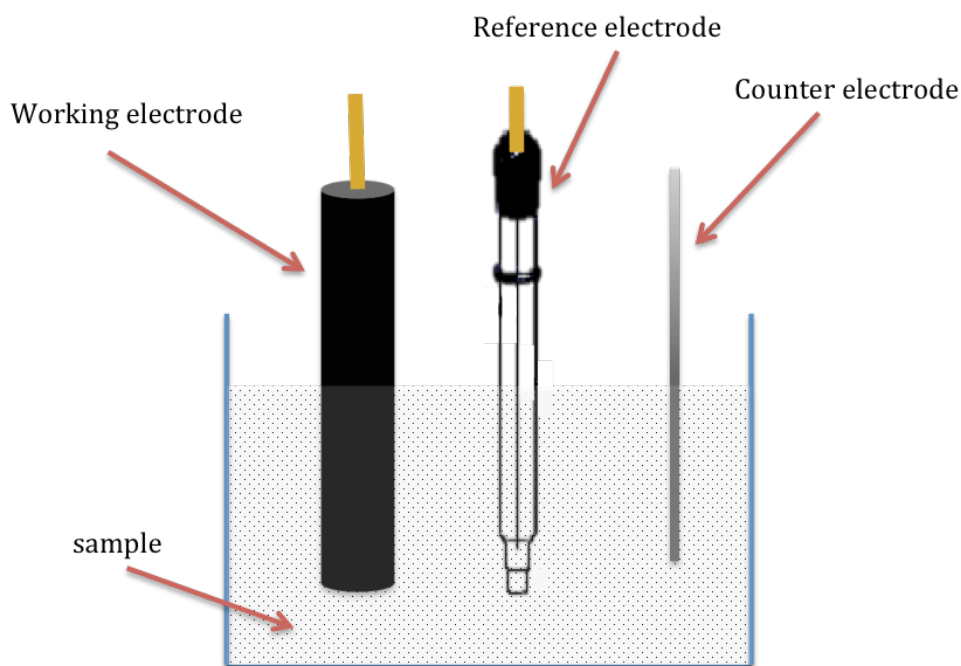


**Figure 5** Cross section of key part of a glucose test strip (adapted from diabetes.org)

Figure 5 displays a cross section of a test strip. The Diabetes Forecast magazine describes the anatomy of test strips in the following way. The top layer is a coating made up of an adhesive material to protect the circuit. Then there are four layers that make up the sample chamber; these are adhesive materials and a spacer. The chamber has a small transparent window that gives the observer a visual indication that the strip has been properly loaded with the blood sample. The next small, yellow layer is where the chemical reaction takes place. The enzyme oxidizes the glucose and transfers an electron to a mediator, which then passes them into the circuit via an electrode. In the meter the electrons are measured as current and it is this measured current which is converted into a concentration of glucose based on a calibration curve.<sup>23</sup>

### 1.2.2 Electrochemical Sensors

Electrochemical sensors are the industry standard because they display low detection limits, have faster response times, and are less expensive than other detection platforms.<sup>24</sup> Electrochemistry implies that there is a transfer of charge from an electrode to another phase (i.e. liquid to solid), both the electrode reactions and/or the charge transport can be chemically controlled and serve as the basis of the sensing process.<sup>25</sup> A fundamental rule that applies to all electrochemical sensors is that they must be a closed circuit, meaning that there is a sensor electrode and a signal return. There are three important parts that make up a three-electrode electrochemical cell: (1) the working electrode, (2) auxiliary (counter) electrode, and (3) the reference electrode.



**Figure 6** A conventional three-electrode electrochemical cell

Figure 6 portrays a typical schematic of a three-electrode electrochemical cell setup with the aforementioned electrodes. Depending on whether the electrons are added to or

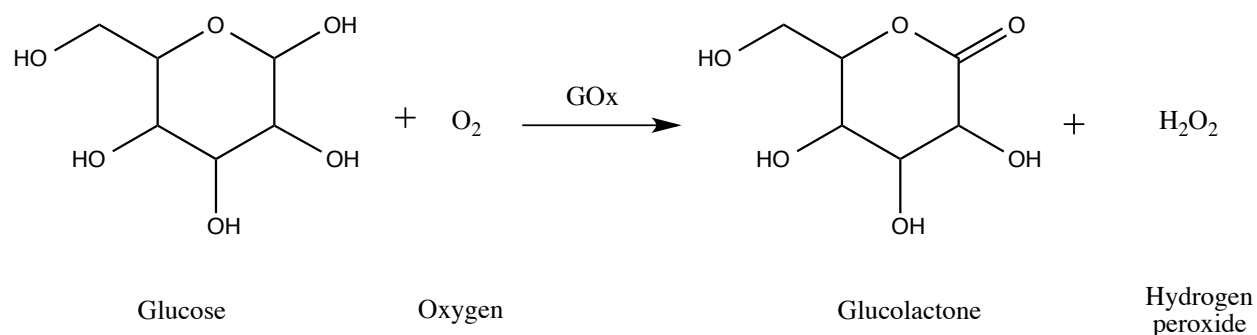
withdrawn from the sample, the working electrode, acts as either an anode (oxidation) or a cathode (reduction). For the working electrode the potential with respect to the reference electrode is varied overtime. The role of a reference electrode is to provide a stable, reversible, and reproducible voltage so that the working electrode potential may be referenced.<sup>26</sup> In other words, it establishes a chemical potential that is constant against other potentials, which can be measured. The counter electrode functions as a cathode when the working electrode is operating as an anode and vice versa, it conducts electrons from the signal source through solution to the working electrode. Counter electrodes have a larger surface area than the working electrode to ensure that the half-reactions occurring does not limit the process occurring at the working electrode.

### ***1.2.3 Chronoamperometry***

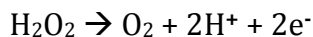
In chronoamperometry (aka amperometry), a constant potential is applied to the working electrode, which results in an oxidation or a reduction at the electrode surface, and the consequential current is measured overtime. This current scales linearly with the analyte concentration at low concentrations. The chemical reaction for a typical glucose measurement is demonstrated in Reaction 1. A voltage is applied to the working electrode vs. a reference electrode and the resulting anodic current shown in Reaction 2 is monitored. It is worth noting that current only flows if the voltage applied is greater than the decomposition voltage. The deposition voltage refers to the minimum voltage required between the cathode and anode for electrolysis to occur.<sup>25</sup>

Amperometric enzyme electrodes have revolutionized the development of biosensors. The history of enzyme electrodes started in 1962 with the development of the

first device by Clark and Lyons, as they gave the first description of the biosensor.<sup>18</sup> The first enzymatic part of the biosensor relied on a thin layer of GOx entrapped on a working electrode via a semipermeable dialysis membrane. The measurements are based on the monitoring the oxygen consumed by the enzyme-catalyzed reaction.<sup>27</sup> The reaction is shown in Figure 6.



**Reaction 1** Reaction in coating above working electrode

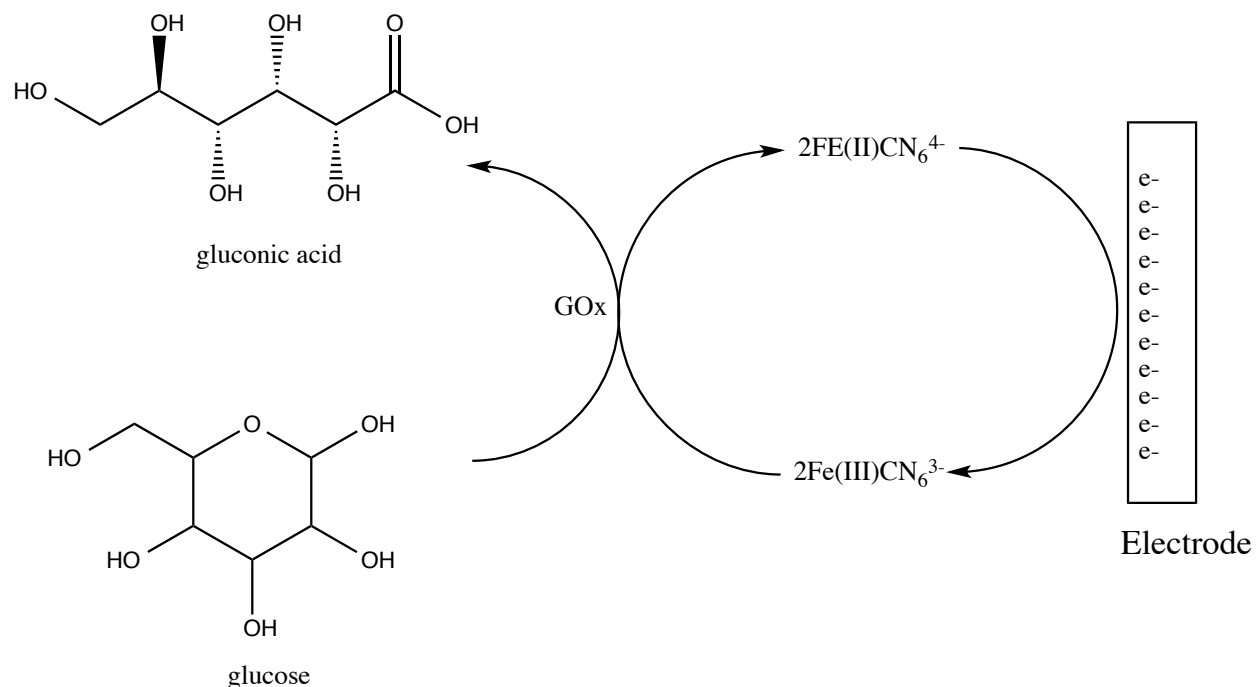


**Reaction 2** Reaction on surface of working electrode

As glucose reacts with oxygen and GOx it yields glucolactone and hydrogen peroxide. Without the GOx the oxidation of glucose is negligible. Glucose biosensors measure the H<sub>2</sub>O<sub>2</sub> by oxidation at the working electrode. Thus, on the surface of the working electrode Reaction 2 is occurring. The current is proportional to the concentration of H<sub>2</sub>O<sub>2</sub>, which in turn is proportional to the glucose concentration in blood.

### 1.2.4 Mediators

A difficulty of the glucose monitor developed by Clark and Lyons was the dependence on the concentration of  $O_2$  in the enzyme layer. The way to reduce the dependence of  $O_2$  is to substitute it with a mediator. Electrochemical measurements often require mediating reagents, which are substances that facilitate or mediate the transfers of electrons. Potassium ferricyanide is a well-known mediator, and it is often used to react with GOx since it facilitates the transfer of a single electron from glucose to the electrode. This particular mediator is reduced to ferrocyanide in the process. The chemical reactions on the electrode surface are depicted in Reaction 3. The glucose in the blood sample reacts with the GOx to form gluconolactone, which then reacts with ferricyanide to form ferrocyanide.<sup>27</sup> The electrode oxidizes the ferrocyanide, and this generates a current that is proportional to the glucose concentration.



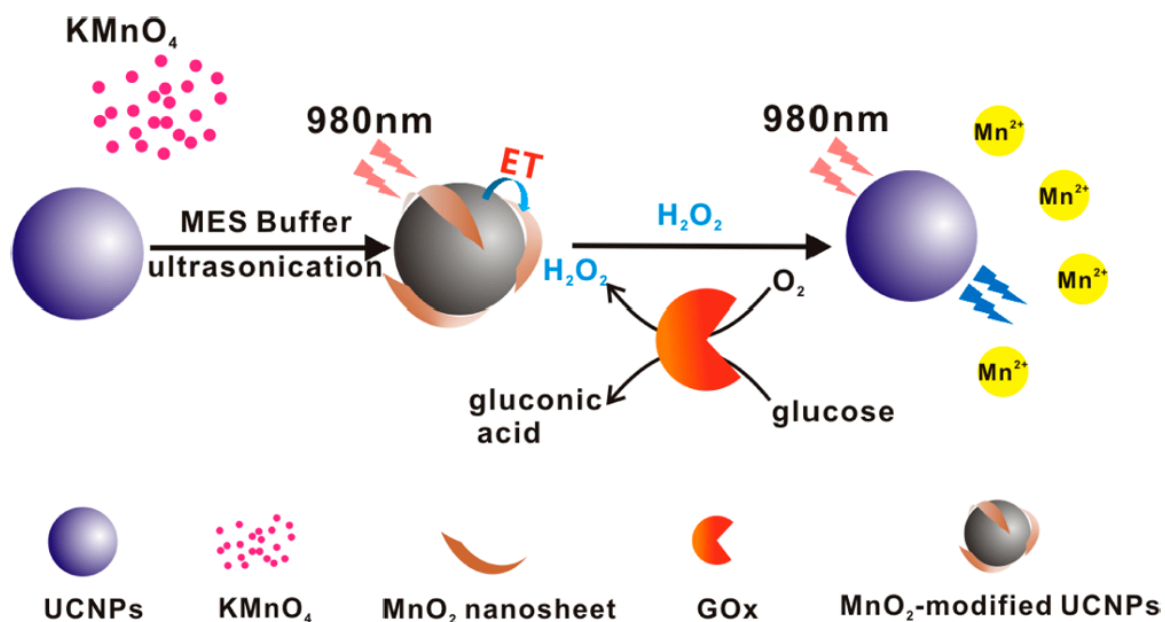
**Reaction 3** Electrode receiving electrons from glucose facilitated by a mediator

### **1.3 Other approaches to measuring glucose concentration**

Due to the growing global epidemic of diabetes, there is an increased demand for glucose sensors that are both biocompatible and have increased sensing capabilities. Thus, there has been intense research geared towards development of non-invasive glucose sensors. This includes nonenzymatic glucose sensors, fluorescence, and prototype wireless circuit biosensors.

Ahmad *et al.* developed a nonenzymatic glucose biosensor based on copper oxide (CuO) nanoparticles inject-printed on silver electrodes. CuO nanoparticles were prepared as ink using a mixture of solvents. Their working electrode was based on a Si/Ag with CuO nanoparticles. They performed cyclic voltammetry measurements using the synthesized electrode to verify workability on human serum, and the calculated results were in accordance with the known concentration of glucose from the serum. This is an innovative method for mass production, as well as for long-term analysis of glucose as an amperometric sensor for blood serum.<sup>28</sup>

Research groups are also working on fluorescence-based glucose detection methods. Yuan *et al.* developed fluorescence detection techniques based on manganese dioxide (MnO<sub>2</sub>) nanosheet-modified upconversion nanoparticles (UCNPs) for fast and sensitive glucose detection in human serum.<sup>29</sup> A sample may fluoresce based on the H<sub>2</sub>O<sub>2</sub> mediated reduction of MnO<sub>2</sub> to Mn<sup>2+</sup>, which leads to the decomposition of the MnO<sub>2</sub> nanosheets. Thus, because glucose can be oxidized by O<sub>2</sub> in the presence of GOx to produce H<sub>2</sub>O<sub>2</sub>, a biosensor can be constructed based on the 'upconversion' fluorescence assay for monitoring blood glucose. Figure 7 below explains this concept.



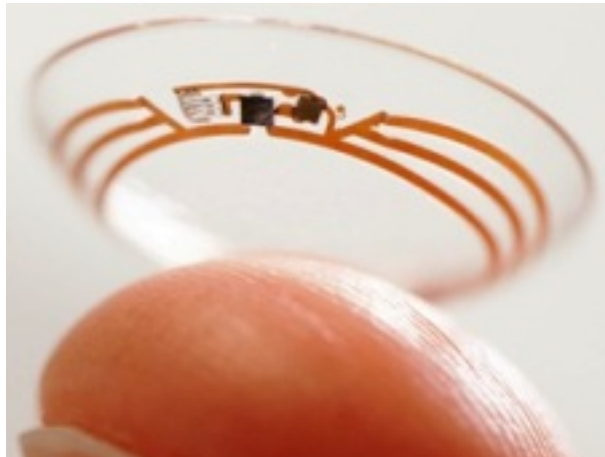
**Figure 7** Design and principle for H<sub>2</sub>O<sub>2</sub> and glucose detection using a MnO<sub>2</sub>-nanosheet-modified UCNP nanocomposite (acquired from Applied Material Interfaces 2015)

Figure 7 illustrates that MnO<sub>2</sub> nanosheets were formed and functionalized onto the UCNP's surface. MnO<sub>2</sub> quenches the upconverted luminescence; this effect is reversed by adding H<sub>2</sub>O<sub>2</sub>, which prompts the reduction of MnO<sub>2</sub> to Mn<sup>2+</sup>. This method shows promise for more sensitive methods and clinical diagnosis in diabetes.

Moreover, the latest noninvasive way to detect glucose has been made by Google along side Novartis, a European drug company. They are working on a Smart Contact Lens Project, which uses contact lenses with a wireless chip and a miniaturized glucose sensor embedded between two layers of soft contact lens material.<sup>30</sup> Figure 8 displays how the prototype might look as a contact lens. This device allegedly measures glucose levels in tears although the mechanism of how this might work is still unknown to the public. This device is still in the prototype phase and various clinical trials and FDA approval are required before it is commercially available. This device might be difficult for some patients



to use because not everyone is accustomed to wearing contact lens and people may even be opposed to inserting a foreign object in their eye. The methodologies used in these studies are very intrusive for the patient; however, the method presented in this project provides a non-invasive approach.



**Figure 8** Smart Constant Lens Project with wireless circuit

#### ***1.4 Measurements of tear glucose concentration***

The usual approach to measuring glucose requires an invasive and sometimes painful methodology by the withdrawal of blood. As an alternative to blood, approaches to measure glucose in easily accessible fluids, such as urine, saliva, and tear fluid have been investigated, as they have great potential for noninvasive diagnosis and management of diabetes.<sup>31</sup> A correlation between tear glucose and blood glucose has been studied, but the results are inconclusive. LeBlanc et al. found a lack of consistent correlation between tear and blood glucose values. However, Meyerhoff et al. reported a correlation between the two. Moreover, other researchers have reported that there is evidence of correlations, but more studies should be conducted to determine the potential utility.<sup>32</sup>

There is still controversy on the correlation between the concentrations of tear glucose in normal and diabetic patients.<sup>33</sup> However, Baca *et al.* concluded that there is evidence indicating a correlation in the average tear and glucose levels in rabbits, but further characterization and measurements are necessary from human studies to determine potential utility of tear glucose measurements. Meyerhoff *et al.* examined the use of commercial glucometer strips that require less than 1  $\mu\text{L}$  to measure glucose in tears. Many research groups have examined the detection methods and limits for measuring levels of glucose in tears.<sup>34</sup> In the 1980s, it was discovered that tear glucose levels were significantly higher in diabetic patients than those in regular patients.<sup>34</sup> However, the tear glucose levels are 30-50 times lower than the blood glucose levels. As such, Meyerhoff describes a needle-type amperometric enzyme electrode for glucose that is capable of measuring the levels of glucose in tear fluid down to 1.5  $\mu\text{M}$  within a capillary tube containing approximately 5  $\mu\text{L}$  of tear fluid. This research group concluded that in rabbit models, there was a significant correlation between tear and blood glucose levels, but the exact correlation varies from rabbit to rabbit.

The blood glucose concentration for patients without diabetes ranges from 70 to 140 mg/dL (3.9-7.8 mmol/L) before meals and peaks about 200 mg/dL (11.1 mmol/L) for up to two hours after a meal. Patients with diabetes can reach a range greater than 240 mg/dL (13.3 mmol/L).<sup>35</sup> In contrast, average fasting glucose concentration in tears were previously measured to be 0.2 mmol/L for non-diabetics and 0.92 mmol/L for diabetics. Table 2 displays data of tear glucose concentrations based on literature from 1980, 2005, 2006, 2007, and 2010, thus confirming that there is glucose concentration in tears.

<b>Table 2: Comparison of non-diabetic vs. diabetic glucose concentration in tears</b>		
<b>Author/ journal/ year</b>	<b>Non-diabetic</b>	<b>Diabetics</b>
La Baella. Journal of Diabetes Science and Tech, 2010. <sup>36</sup>	0-0.28 mmol/L (5-20 mg/dL)	N/A
Baca, J. The Ocular Surface, 2007. <sup>33</sup>	0-3.6 mM (65 mg/dL),	4.7 mM (84 mg/dL)
Lane J.D. Current Eye Research, 2006. <sup>39</sup>	0.35 ± 0.04 mmol/L (6.34 ± 0.7 mg/dL)	0.16 ± 0.03 mmol/L (2.9 ± 0.5 mg/dL)
LeBlanc J.M. Intensive Care Med., 2005. <sup>37</sup>	7.25 ± 5.47 µmol/L	N/A
Sen, D.K. British Journal of Ophthalmology, 1980. <sup>38</sup>	0.2 mmol/L (3.6 mg/dL)	0.92 mmol/L (16.6 mg/dL)

In 2014, Cha's research team at the University of Michigan published an article examining commercial glucometer test strips for the measurement of glucose in tears.<sup>32</sup> They understood that regular measurements and control of blood glucose was important to avoid life-threatening hyper and hypoglycemic events and side effects that were associated with long-term complications. Some of the complications are: retinopathy, nephropathy, neuropathy, macrovascular disease, and amputations. For optimal control of glucose levels a patients must check their glucose at least four times a day. However, children and adolescents with type 1 diabetes on insulin therapy must check their glucose levels up to eight times a day.<sup>39</sup> The discomfort that can arise from monitoring glucose levels so many times a day limits patient compliance to monitor and regulate their glucose levels. Thus, with this in mind Cha *et al.* tested how well different commercial test strips measured the concentration of glucose. It was concluded that ACCU-CHEK Aviva Plus glucometer exhibit the sensitivity and selectivity necessary to measure glucose concentration in tears. In addition, Cha suggests that methods for measuring tear glucose must have low micromolar

range limit of quantification (LOQ), high selectivity over interferences, and measurement potential within microliter sample volumes.<sup>32</sup>

### ***1.5 Objective***

The development of a non-invasive and pain-free technique for measuring glucose concentration will be beneficial for patients with diabetes. The goal of this project is to find a noninvasive way to monitor their glucose levels without the discomfort and painful pricking of fingers. This may help decrease the risk of the complications that may surface as a result of not monitoring glucose levels and increase patient compliance. To control blood glucose levels, regular monitoring is required. We aim to use tear glucose as an alternative for the discussed blood glucose measurements. In order to do this, we must first test if there is a correlation between the tear glucose and blood glucose concentrations. We used commercially available glucometers to test blood glucose concentration and a home-built alternative biosensor, the tearometer, using the electrochemical technique of chronoamperometry. We hope to optimize the biosensor device by measuring the sensitivities and limits-of-detection to accurately measure tear concentrations.

## ***Chapter 2: Experimental***

### ***2.1 Chronoamperometry experiments***

Chronoamperometry measurements were carried out using a Potentiostat, CHInstruments (CHI) Model 604E *Picoamp Booster & Faraday Cage* and the CHInstruments *Electrochemical Analyzer* (Figure 9 a), this was controlled by a computer via CHInstruments software. All measurements were obtained at room temperature.

#### ***2.1.1 Preparation of glucose***

The 1 M glucose stock solution with DI water was be prepared at least 24 hours prior to performing the experiments and stored in a dark refrigerator at 4°C in order for the glucose to mutarotate<sup>40,41</sup> to  $\beta$ -D-glucose. From this stock solution, several dilutions were made and used: 100 mM, 10 mM, 1 mM, and 0.1 mM of glucose.

#### ***2.1.2 Preparation of phosphate buffer and glucose oxidase***

A 10 mM phosphate buffer, 0.1 M NaCl stock solution adjusted to pH 7.0 was obtained.

Glucose oxidase (GOx) from *Aspergillus Niger* (17,300 units g<sup>-1</sup>), was obtained from Sigma-Aldrich; 2000 units of GOx was mixed with 100  $\mu$ L of the 10 mM phosphate buffer (pH 7.0) and stored at 4°C.

### ***2.1.3 Preparation of dialysis membrane***

A 10 cm long fragment of dialysis membrane (MWCO 12,000) was boiled in a 1% w/v sodium carbonate solution for 10 minutes and then it was stored in 0.01 M Tris buffer containing 1 M EDTA at 4°C. The dialysis membrane was soaked in 10 mM phosphate buffer for a minimum of 5 minutes before using.

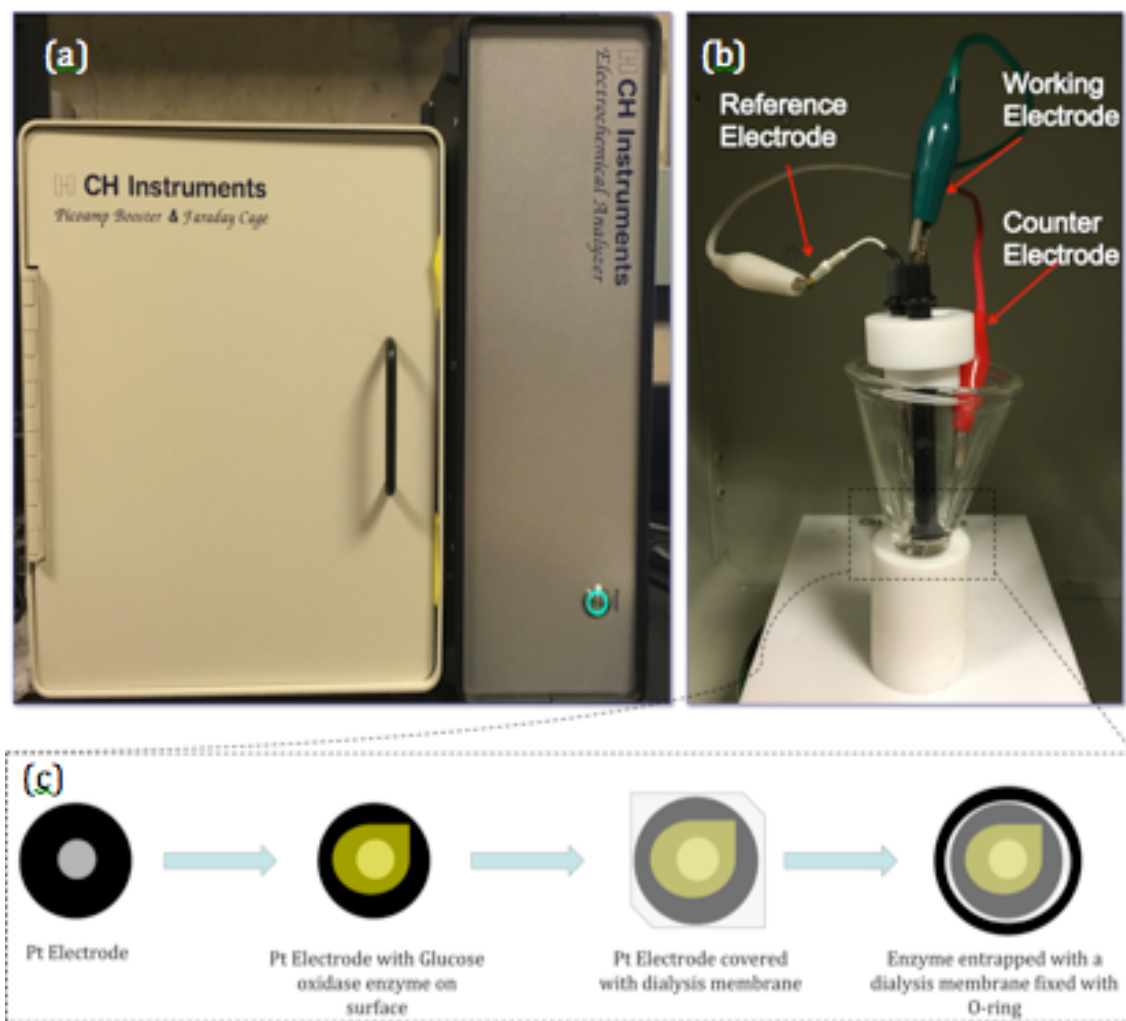
### ***2.1.4 Polishing the electrodes***

The electrodes were polished with a CHI electrode polishing kit. The working electrode was a 3 mm diameter Pt electrode; it was rinsed with DI water, then methanol, and wiped dry. Three alumina grits were used, 1  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , and 0.1  $\mu\text{m}$ , sequentially. The alumina suspension was dispensed evenly on the surface of the pad. The working electrode was placed faced down on the nylon microcloth pad, and the electrode is moved in a circular motion all over the pad. After  $\sim 1$  minutes, the electrode was removed and rinsed with DI water.

### ***2.1.5 Preparation of electrochemical cell***

A platinum (Pt) disk-working electrode was used for all of the experiments performed. Figure 9(c) shows the surface where the electrochemical reaction takes place. A pipette was used to place 5  $\mu\text{L}$  of GOx on the clean surface of the Pt working electrode. A 2x2  $\text{cm}^2$  piece of dialysis membrane was carefully placed on top on the electrode surface. The membrane was fixed by an O-ring and submerged in a 10 mL of phosphate buffer. Figure 9c shows the surface of the working electrode. The platinum counter electrode and the

Ag/AgCl reference electrode were also submerged in the buffer and connected to potentiostat for analysis (Figure 9b).



**Figure 9** (a) the potentiostat. (b) the electrochemical setup with working electrode (green), reference electrode (white) and counter electrode (red). (c) Surface of the working electrode where the enzyme catalyze chemical reaction takes place

For experiments that used larger volumes of buffer (e.g. 10 mL, 5 mL) a shot-glass shaped container is used. For experiments that use small volumes of buffer (e.g. 200  $\mu$ L) a coned-shaped shot glass is used as shown in Figure 9.

### 2.1.6 Chronoamperometry parameters

The chronoamperometry parameters used are shown below.

Table 3: Parameters	
Initial Voltage (V)	0
High Voltage (V)	0.652
Low Voltage (V)	0.65
Initial Step Polarity	Positive
Number of Steps	1
Pulse Width (sec)	120
Sample Interval (sec)	1
Quiet Time (sec)	2
Sensitivity (A/V)	$1 \text{ e}^{-005}$

### 2.2 Collection of blood and tears

The procedure for the measurement and collection of blood and tears was approved by the Institutional Review Board. The verbal consent to participate in the experiment, the consent to withdraw and measure blood, the preliminary questions, and advertising poster can be found in the supplemental information.

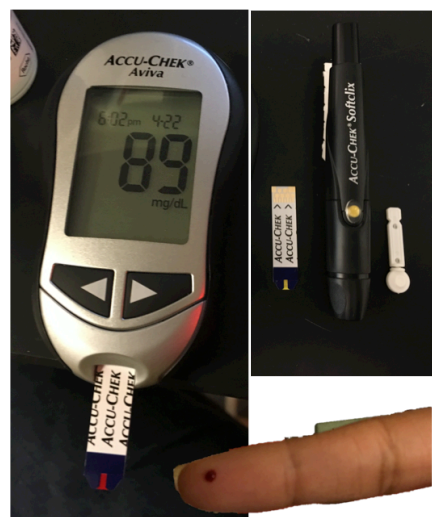
#### 2.2.1 Procedure for performing blood glucose tests

Step 1: The subject washes their hands with soap and water.

Step 2: The meter is turned on. After 3 seconds, the test strip is inserted into the glucometer in the direction of the arrows.

Step 3: On the meter screen, a test strip and a flashing blood drop appear on the display.

Step 4: The lancet device is loaded with the lancet and ready for use.





Step 5: The subject uses the lancet device, aims it at their finger and gets a blood drop.

Step 6: The blood drop is placed to the front edge of the window on the test strip.

Step 7: The blood glucose concentration is displayed on the screen.

Step 8: The test strip is taken out of the meter and the lancet from the lancing device and properly disposed.

Step 9: The person must wash their hands again and place a band-aid over affected area.

### ***2.2.2 Procedure for obtaining tears***

To induce tears, the participant used a commercially available lachrymator, Kryolan Tear Stick, which contains menthol. The Kryolan was rubbed 15 millimeters under the eye, the warmth from the skin released menthol vapors, which induces tear production. The tears (~200  $\mu\text{L}$ ) were collected in a micro-centrifuge tube after the blood glucose level was measured.



(Left) A participant applying Kryolan tear stick under the eye to induce tears. (Right) Participant collecting their tears in micro-centrifuge tube.

### ***2.2.3 Chronoamperometry measurements***

For a typical calibration curve a shot-glass containing 10 mL of phosphate buffer equipped with a micro stir bar was used. The potentiostat was set up with the appropriate parameters and after the electrochemical cell was ready for use. The enzyme was conditioned by applying a constant voltage (0.65 V) to the working electrode in a buffered solution for 15 minutes. This data was saved as buffer with [0] glucose. Next, 10  $\mu$ L of the 1 M (100 mM, 10 mM, 1 mM, or 0.1 mM) glucose stock solution was added to the shot-glass and stirred for exactly 2 minutes. Next, the shot-glass was placed back into the Faraday cage and the amperometry data was measured for 2 minutes. Subsequently 10  $\mu$ L of glucose was added again, and these last steps were repeated several times.

For a glucose calibration curve with human tears a cone-shaped shot glass was used. The potentiostat was set up with the appropriate parameters and subsequently the electrochemical cell was set up, the enzyme was conditioned in the tears for 10 minutes. The data collected was saved as tear buffer with [0] glucose. Next, 10  $\mu$ L of 1 mM glucose solution is added to the shot-glass and mixed with a pipette. The amperometry data was measured and saved; the current at the end of the 2 minutes was collected and graphed. Then, 10  $\mu$ L of glucose was added again, and these last steps were repeated several times until a calibration curve was obtained.

### ***2.2.4 Calibration curve for precision and limit of detection***

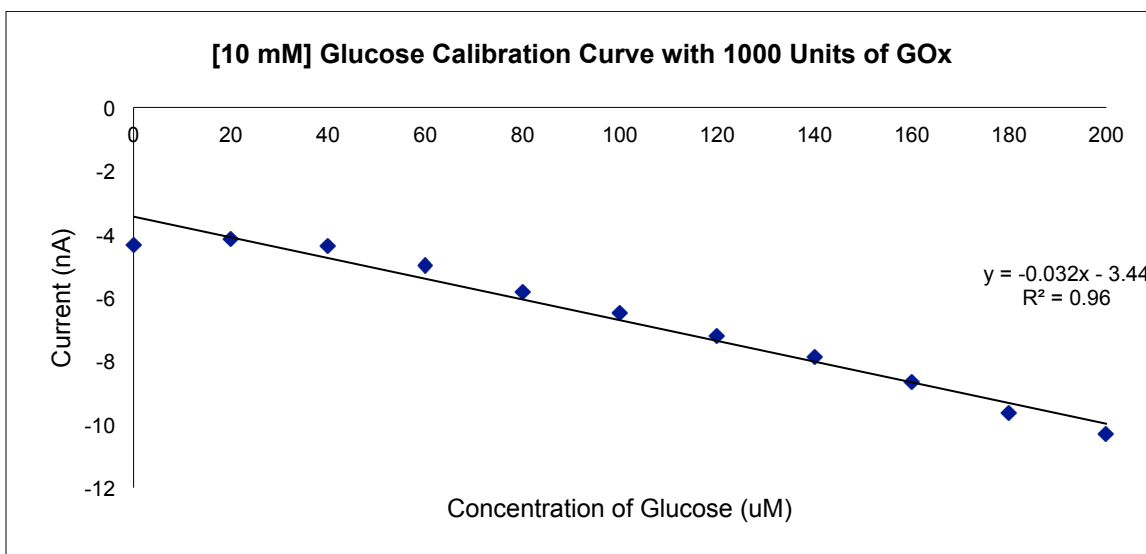
To measure the precision and limit of detection of the tearometer 5 mL of phosphate buffer was used in a shot glass. A 250  $\mu$ L aliquot of the 100 mM concentration glucose solution

was added to the buffer and stirred for 2 minutes, after which the current was monitored and recorded. Next, 20  $\mu\text{L}$  of 1M glucose solution were added and stirred for two minutes, then the current was recorded. This was done 4 more times, until a calibration curve was obtained. This experiment was repeated five times.

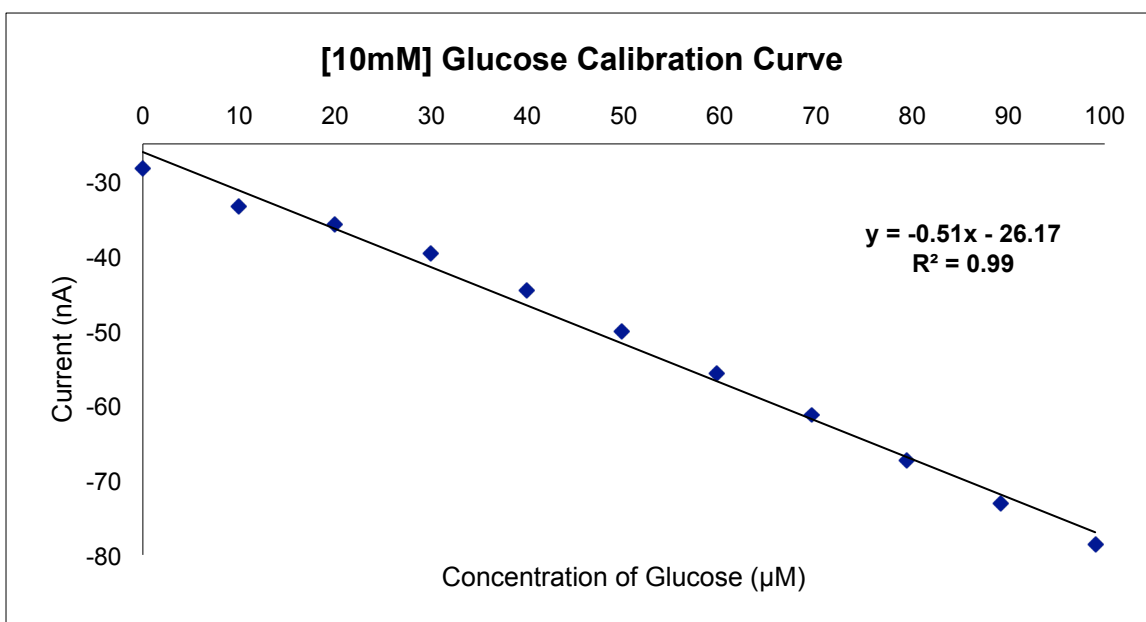
## ***Chapter 3: Results and Discussion***

### ***3.1 Optimizing the biosensor***

In experimental design we aim to find the conditions to optimize the biosensor performance. Before we use the tearometer, for tear measurements we tested different parameters to ensure the system's ideal performance. The rate of the enzymatic reaction can be varied by changing the enzyme concentration substrate concentration or the reaction temperature.<sup>42</sup> One of the parameters we sought to optimize was the concentration of GOx, which is at the heart of the biosensor.<sup>43</sup> Initially we used 1000 units of GOx and performed several measurements. Figure 10 shows the curve obtained from a 10mM glucose solution. The trend-line acquired from the graph does not fit all of the points. The first 40  $\mu\text{L}$  added do not seem to change the signal, but after 60  $\mu\text{L}$  the points seem to fit the trend-line, which had an  $R^2$  value of 0.96. We wanted detect very small concentrations of glucose, thus we tried 2000 units of GOx instead of the 1000 units. Figure 11 demonstrates a 10 mM glucose calibration curve using 2000 units of GOx. We were able to clearly see a difference in signal from the first addition of 20  $\mu\text{L}$  of glucose and the  $R^2$  value was 0.99. Thereafter we used 2000 units of GOx. This larger concentration of GOx results in faster reaction rates and enhances the production of hydrogen peroxide. Thus, for optimal conditions of the biosensor every measurement performed used 2000 units of GOx.



**Figure 10** [10 mM] glucose calibration curve in aliquots of 20  $\mu$ L in 10 mL phosphate buffer



**Figure 11** [10 mM] glucose calibration curve using 2000 units of GOx by adding 20  $\mu$ L aliquots in 10 mL phosphate buffer

### **3.2 Precision**

The ACCU-CHEK Aviva Plus System was used to measure blood glucose concentration in human subjects. We also attempted to measure tear glucose concentration with the glucometer device since Meyerhoff et al. suggested that this would be the best glucometer for samples with low concentration such as tears. However, after several trials, we found that this device could not measure tears; on the display screen it read LO' indicating that the concentration was too low to be detected by the glucometer. Thus, we wanted to find the detection limit of the device so we ran trials of known glucose concentrations and found that the glucometer could only measure down to 1 mM glucose solutions, when one tries to measure 0.1 mM glucose, the concentration is too low to measure. Therefore, the limit of detection of the glucometer is between 1 mM and 0.1 mM, which is in agreement with the manufacturer's specification of 20 mg/dL (1.11 mM).

In order to measure the precision of the glucometer several glucose measurements were taken on a 1 mM glucose sample. The same sample solution was measured ten times and the following readings were obtained: 236, 256, 258, 270, 282, 287, 290, 294, 299, and 300 mg/dL. The average measurement and standard deviation were calculated to be  $277 \pm 21$  mg/dL (15.4 mmol/L) in the timespan of 5 minutes. We found a 7% error with the glucometer. This percentage may not seem significant, but this difference may be essential to a patient with diabetes. The range of values may be due to strip-to-strip variation, and large difference in the average value and the expected value is likely due to matrix effects. The matrix of our standard was DI water whereas the strips are calibrated to test blood

samples, which accounts for the increasing difference in the measurements between whole blood and glucose solutions.<sup>17</sup>

Measuring glucose in tears is difficult for two reasons: i) we are only able to collect small volumes of tears (200-250  $\mu\text{L}$ ) and ii) the glucose concentration is significantly lower in tears (0.92 mmol/L) than blood ( $\sim 1$  mmol/L). To overcome the first problem we gradually decreased the volume of the buffer we use to make measurements. To be sure the tearometer could deal with the second problem we measured the precision, limit of detection, and sensitivity of the tearometer to investigate the feasibility for the detection of glucose concentrations in tears.

To evaluate the precision of the tearometer we measured the concentration of a 100 mM glucose sample several times. We used either 10 or 5 mL of buffer and added 250  $\mu\text{L}$  of the glucose concentration that we wanted to measure. After measuring a baseline current, we performed standard addition by adding 20  $\mu\text{L}$  of 1 M glucose solution. The current was measured at 2 minutes after every addition and then graphed to create a calibration curve. This experiment was repeated four more times to compare the calculated concentration of the 250  $\mu\text{L}$  of 100 mM glucose solution. A total of five measurements were performed and the following concentrations were calculated: 152, 97, 135, 152, and 91 mM. The average measurement and standard deviation were computed to be  $125 \pm 29$  mM. The theoretical glucose concentration was 100 mM, but the measured value was 125 mM. The tearometer had a 25% error in these measurements.

### 3.3 Limit of detection

As a means to measure the limit of detection of the tearometer we added a known concentration of glucose solution into a phosphate buffer solution (10 mL or 5 mL) and examined the current as it responded to each new volume of glucose added. We started with high concentrations of glucose solutions such as 1 M, 100 mM, and 10 mM. The standard addition calibration curves for the aforementioned concentrations are linear with  $R^2$  values of 0.99. The 10 mM glucose concentration standard addition graph, Figure 11, shows a trend-line that fits most of the points very well. For this experiment we added 20  $\mu\text{L}$  of 10 mM glucose solutions to a 10 mL phosphate buffer, thus diluting the small aliquot of glucose solution to the buffer and obtaining a signal that followed the trendline. This signal indicates that the tearometer can measure a change in concentration of 20  $\mu\text{M}$  of glucose solution. Next, we examined 1 mM of glucose solution in 5 mL of phosphate buffer. Figure 12 demonstrates that the linearity of the standard addition diminishes; this is confirmed by the  $R^2$  value of 0.97. However, we are able to detect a lower concentration of changes in glucose at 4  $\mu\text{M}$  as it is diluted in the 5 mL buffer.

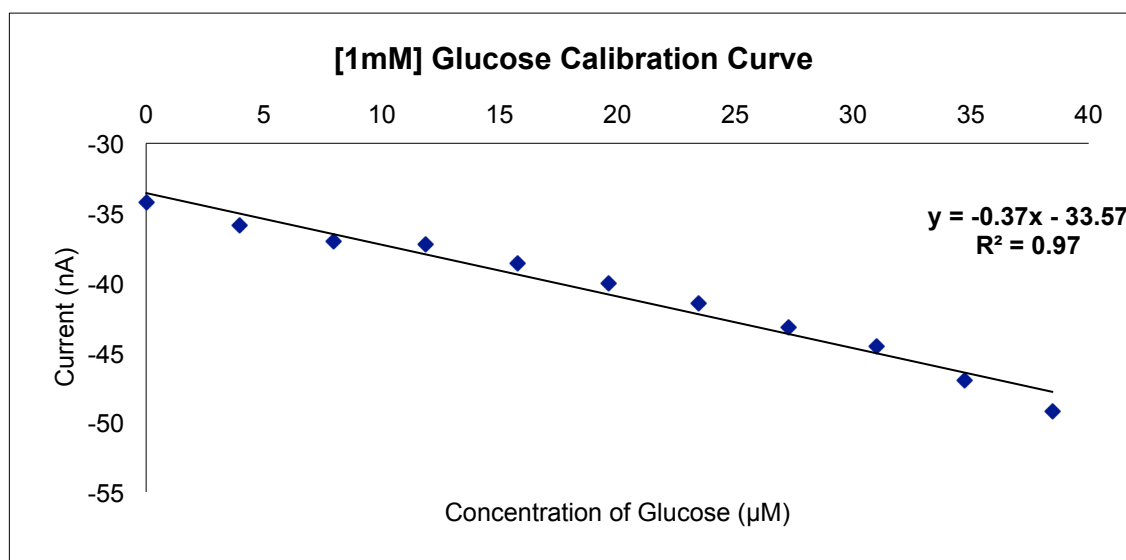
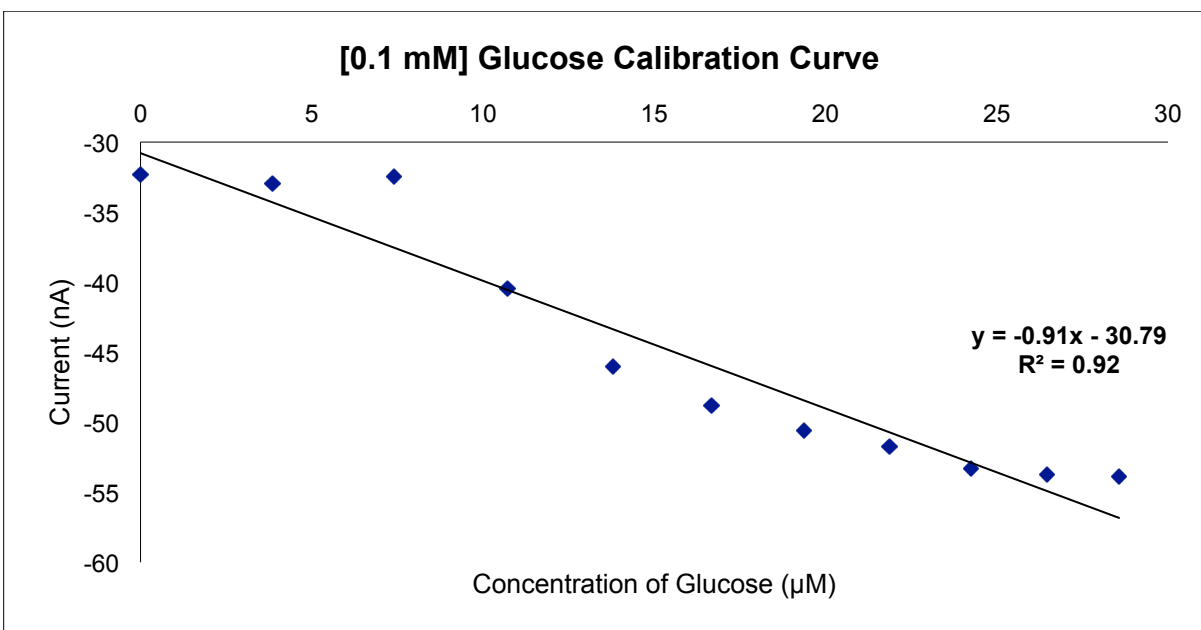


Figure 12 [1 mM] glucose calibration curve in aliquots on 20  $\mu\text{L}$  in 5 mL phosphate buffer





**Figure 13** [0.1 mM] glucose calibration curve in aliquots on 20 μL in 500 μL phosphate buffer

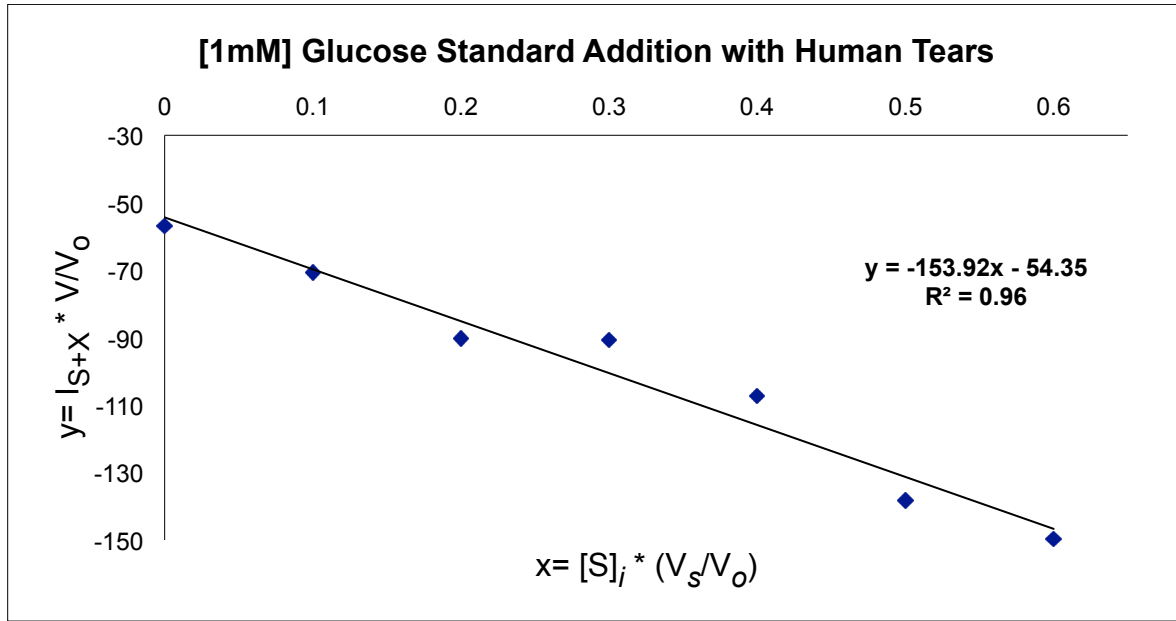
Lastly, to continue testing the limits of detection we used a 0.1 mM glucose solution in a 500 μL buffer. As we decrease the glucose concentration more discrepancies are seen in the graph's linear fit. This is confirmed by the  $R^2$  value of 0.92 in Figure 13.

Based on these graphs we conclude that the limit of detection is approximately 20 μM. When we analyze Figure 10, the [10 mM] calibration curve, we see that when the concentration of the solution is ~20 μM the points fit the trend-line almost perfectly. The same is true in the Figure 11. When concentrations lower than 20 μM were measured the data did not fit a line well and so we do not have confidence in these measurements. At 20 μM we are able to easily discriminate between the concentration obtained from the measurement and the blank sample and we should be able to measure the glucose concentration in tears since according to literature it is in the 200 uM range (Table 2), which is approximately 10 times higher than our current detection limit.

In contrast to the limit of detection where we try to measure low levels of glucose, the sensitivity of the tearometer can be analyzed by the difference in signal when a small volume of glucose is added to the solution. Thus, according to the 1 mM calibration curve (Figure 12), the tearometer has a sensitivity of 0.34  $\mu\text{M}/\text{nA}$ , which enabled us to detect changes in glucose concentration of 4  $\mu\text{M}$  when the standard was added.

### ***3.4 Measuring glucose concentration in tears***

We next proceeded to measure the glucose concentration in human tears with confidence that the tearometer would be able to detect the low concentrations of glucose. Instead of our usual 5 mL of phosphate buffer to which we would normally add a standard glucose solution, we used the tears themselves for the baseline measurements and added aliquots of 1 mM glucose solution for the calibration curve. When the tear sample was collected, we also measured the blood glucose concentration of the same individual in order to test if there was a correlation between the glucose levels.



**Figure 14** Measurement of tear glucose levels via standard addition when blood glucose concentration was 91 mg/dL

In order to calculate the glucose concentration in the tear we use the standard addition equation:

$$\frac{[X]_i}{[S]_f + [X]_f} = \frac{I_X}{I_{S+X}} \quad (eq. 1)$$

In standard additions a sample with unknown initial concentration of analyte,  $[X]_i$ , gives a signal intensity  $I_X$ . Then a known concentration of standard,  $S$ , is added to an aliquot of the sample and a signal,  $I_{S+X}$ , is observed for the second addition. As expected the addition of the standard to the unknown changes the concentration of the original analyte both because of the additional analyte and because of dilution. The diluted concentration of the analyte is  $[X]_f$  and the concentration of the standard in the final solution is  $[S]_f$ . The signal is directly proportional to analyte concentration, which gives equation 1. Equations 2 and 3 show the dilution factors, which relate the final concentration to initial concentration.<sup>44</sup>

$$[X]_f = [X]_i \left( \frac{V_o}{V} \right) \text{ (eq. 2)}$$

$$[S]_f = [S]_i \left( \frac{V_s}{V} \right) \text{ (eq. 3)}$$

eq. 1 is rearranged:

$$I_{S+X} [X]_i = I_X ([S]_f + [X]_f)$$

substitute eq. 2 and 3 into eq. 1

$$I_{S+X} [X]_i = I_X \left( [S]_i \left( \frac{V_s}{V} \right) + [X]_i \left( \frac{V_o}{V} \right) \right)$$

$$\frac{I_{S+X} [X]_i}{[X]_i} = \frac{I_X \left( [S]_i \left( \frac{V_s}{V} \right) \right)}{[X]_i} + \frac{I_X \left( [X]_i \left( \frac{V_o}{V} \right) \right)}{[X]_i}$$

$$I_{S+X} = \frac{I_X}{[X]_i} \left( [S]_i \left( \frac{V_s}{V} \right) \right) + I_X \left( \frac{V_o}{V} \right)$$

$$\frac{I_{S+X}}{\left( \frac{V}{V_o} \right)} = \frac{I_X}{[X]_i} \left( [S]_i \left( \frac{V_s}{V} \right) \right) \left( \frac{V}{V_o} \right) + I_X \left( \frac{V_o}{V} \right) \left( \frac{V}{V_o} \right)$$

Thus we obtain the successive standard addition to one solution equation:

$$\underbrace{I_{S+X} \left( \frac{V_o}{V} \right)}_{\text{function for y-axis}} = I_X + \underbrace{\frac{I_X}{[X]_i} \left( [S]_i \left( \frac{V_s}{V_o} \right) \right)}_{\text{function for x-axis}} \quad \text{(eq.4)}$$

Equation 4 now resembles that of a linear equation,  $y = mx + b$ , where the  $Y$ -axis is  $I_{S+X} \left( \frac{V_o}{V} \right)$ , and the  $X$ -axis is  $\left( [S]_i \left( \frac{V_s}{V_o} \right) \right)$  and the  $X$ -intercept is  $[X]_i$ . Thus, we used the standard addition equation 4 to calculate the unknown glucose concentration,  $[X]_i$ , of the tear from the graph. The trendline equation was used to find the  $x$ -intercept when  $y=0$ . Hence, we find that for this tear sample in Figure 13, the concentration is 0.353 mM. The calculation is the following:

*For successive standard additions to one solution:*

$$I_{S+X} \left( \frac{V_o}{V} \right) = I_X + \frac{I_X}{[X]_i} \left( [S]_i \left( \frac{V_s}{V_o} \right) \right)$$

$$y = -153.92x - 54.35$$

*When  $y = 0$*

$$54.35 = 153.92x$$

$$0.353 = x$$

Hence, when the blood glucose level of the person was 91 mg/dL (5.05 mM) their tear glucose concentration was 0.353 mM. This measurement is in agreement with the literature's suggested order of magnitude for tear glucose concentration.

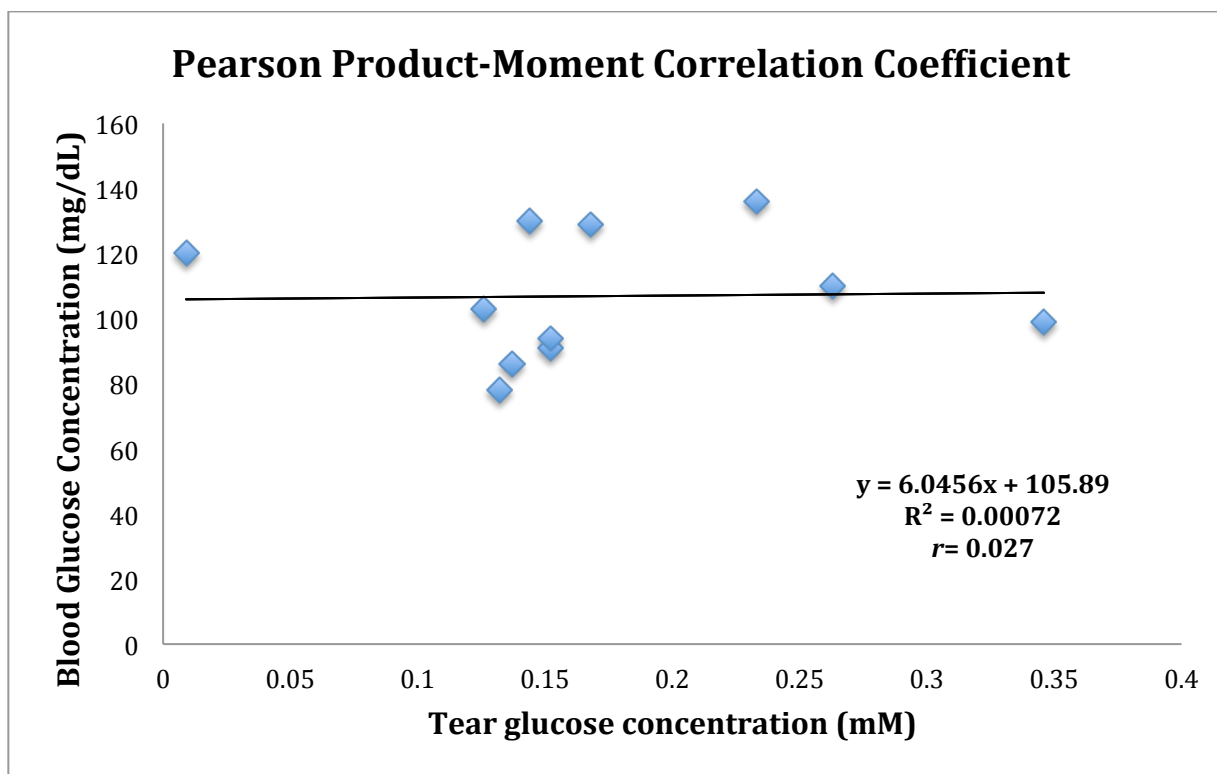
### ***3.5 Correlation between Blood and Tear Glucose Levels***

The pearson product-moment correlation coefficient (PPMCC) is a measure of the linear association between two variables. The two variables are treated equally, thus, either can be the dependent or independent variable. In short, a PPMCC attempts to draw a line of best fit through the data points of the two variables. A Pearson coefficient,  $r$ , indicates how

well the data points fit the trend-line.<sup>45</sup> The strength of association is based on the Pearson correlation coefficient. The stronger the correlation between two variables, the closer  $r$  will be to +1 or -1 depending if it is a positive and negative relationship, respectively. The closer  $r$  is to 0, the greater the distinction around the best-fit line. A value of 0 indicates that there is no correlation between the two variables. The Pearson's correlation makes the following assumption about the variables:

- The variables are either interval or ratio measurements
- The variables are approximately normally distributed
- There is a linear relationship between the variables
- Outliers have either been removed or minimized
- There is homoscedasticity (a sequence of random variables) of the data

The graph in Figure 15 is that of tear glucose concentration vs. blood glucose concentration. Eleven trials were performed to determine the tear glucose concentration after measuring blood glucose levels. The graphed  $R^2$  value is 0.00072. Pearson's coefficient,  $r$ , can be obtained by finding the square root of  $R^2$ , and the calculated  $r$  is 0.027. This value is very close to 0 and therefore indicates that there is no correlation between blood and tear glucose levels.



**Figure 15** Pearson's correlation coefficient, defines the association between two variables. The  $r$  value suggests that there is no association between tear glucose and blood glucose levels.

### 3.6 Sources of error

There are multiples sources of error for this investigation. Only eleven human subjects were used to compare the blood and tear glucose concentration. A greater amount of subjects would give the results more validity by reducing the effects of error. One of the main problems arose from the collection of tears. Although some subjects' tears were easily induced, for others the Kryolan tear stick had no effect and the sample took longer to obtain. Some tear samples were analyzed immediately, while others were analyzed ~30 minutes after they were collected. The time between transport and when the sample is analyzed may be a cause for the discrepancies observed. In addition, we did not use a buffer for the standard additions when determining tear glucose concentrations. Instead

we used  $\sim 200$   $\mu\text{L}$  of tears and added 20  $\mu\text{L}$  of 1 mM glucose solution, which may have minimized the matrix effects on the signal acquired, but may have perturb the tear matrix. Moreover, each tearometer is slightly different from one another despite our best efforts in trying to prepare them the same way, which could be problematic when comparing different tears samples.



## ***Chapter 4: Conclusion***

The rapidly increasing population and style of living have created a need for the development of biosensors for detecting and monitoring diseases with promptness, accuracy, and convenience. Epidemical diseases such as diabetes can constrain the way of life of an individual. As such, helping diabetic patients manage their disease by providing minimally invasive measurements of blood glucose levels is important. This thesis aimed to develop a non-invasive and pain free technique for patients with diabetes to measure their glucose levels. We report the ability to measure glucose concentration in human tears with a homebuilt biosensor, the tearometer. We optimized the biosensor device to reliably measure glucose concentrations in tears. The tearometer has a limit of detection of approximately 20  $\mu\text{M}$ , which is below the report tear concentrations, and a sensitivity of  $\sim 4$   $\mu\text{M}$ . This device has a precision of 25% error. With the tearometer, we were able to successfully measure the glucose concentration in tears, but we were unable to find a correlation between tear and blood glucose levels. This limits the clinical utility of the non-invasive method presented in this investigation of using tears as an alternative for blood in glucose monitoring technique for diabetes.

## ***Chapter 5: Supplemental Information (IRB)***

### ***5.1 Consent for participating in the experiment***

Generally, a person with type I diabetes is required to regulate and monitor the levels of glucose in the blood daily, by pricking their fingers, or else they will not survive. A person going through insulin therapy is required to monitor their glucose levels several times a day. This process can be painful because it requires the person to get pricked by a lancet or even a needle daily. Therefore, the development of a non-invasive and pain free technique can be beneficial for patients with diabetes. To solve this problem we want to make tear glucose measurements and inspect if there is correlation between blood glucose and tear glucose. If the study confirms that there is a correlation, then diabetics will no longer have to endure the daily pains of pricking their finger, instead they will just have to provide a tear, which can easily and non-invasively be provided. The participant will only have to participate one time.

The blood sample will be compared to the participants' tear samples using electrochemical measurements to see if there is a correlation with the concentration of glucose in the samples. Participants will be asked to donate a few drops of blood from the capillaries of their fingers, which can be safely collected by them pricking themselves with a lancet, which is usually used at home by diabetic patients. The droplets of blood will be placed on a strip that goes into a glucometer. I will then collect tears from the same participants by using a commercially available lachrymator, Kryolan Tear Stick. The Kryolan will be rubbed 15 millimeters under the eye, the warmth from the skin will release menthol vapors, which will rise to the eyes and cause tears to come out. Then the samples will be collected on a strip and the glucose concentration will be measured. The lancet may hurt. There is a small risk of bruising. You will not be paid for taking the time to donate a blood sample. You will not be charged for the blood sample.

Some risks that the participant might experience are emotional or slight physical discomfort. Benefits include the satisfaction from contributing to scientific knowledge and candy will be provided.

If you decide not to participate at any moment, it will not affect your job standing, class standing, grades, status on an athletic team, or our friendship.

You can always talk to the study investigator about any questions or concerns you have about this study. Contact the study investigator(s) Ayda Gonzalez at (347) 337-6103 and by email [ag0981@bard.edu](mailto:ag0981@bard.edu). If you have any questions about your rights as a participant in a research project or for more information on how to proceed should you believe that you have been injured as a result of your participation in this study you should contact the study investigator.

Participants will be recruited on a volunteer basis. A flyer will be posted in the RKC building and I will send an email to students in the Chemistry department explaining the project and encouraging volunteers.

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Name

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Signature/ Date

## **5.2 Verbal Consent**

### **Donation of Blood Sample for Laboratory Research Consent to Participate**

Study Title: Correlation Between Blood Glucose and Tear Glucose Levels

Study Investigator: Ayda Gonzalez (student) and Christopher LaFratta (Advisor)

#### **Study Purpose**

In this study, the researcher, Ayda Gonzalez will be measuring blood glucose levels and tear glucose level with a commercially available glucometer. About 30 people will give blood samples for this research. More than 347 million people worldwide have diabetes and this number only keeps increasing. A person with type I diabetes is required to regulate and monitor the levels of glucose in the blood daily, or else they will not survive. A person going through insulin therapy is required to monitor their glucose levels up to 8 times a day. This process can be painful because it requires the person to get pricked by a lancet or even a needle daily. Therefore, the development of a non-invasive and pain free technique can be beneficial for patients with diabetes. To solve this problem we want to make tear glucose measurements and inspect if there is correlation between blood glucose and tear glucose. If the study confirms that there is a correlation, then diabetics will no longer have to endure the daily pains of pricking their finger, instead they will just have to provide a tear, which can easily and non-invasively be provided.

You are being invited to participate in this blood and tear sample donation study. This study is being conducted by Ayda Gonzalez, under the supervision of Dr. Christopher LaFratta.

Donation of blood for research is voluntary and you should not be placed under any pressures to do so. You do not have to agree to give a blood sample nor need to explain why you should choose not to donate. Any personal information provided by you in connection with the donation will be held in confidence. Before the experiment is done there will be preliminary questions, these questions will be confidential, but if any of the answers are no, the participant will not participate in the study.

**For reasons of safety, you should NOT donate/volunteer and are excluded from this study if:**

- You know, or think that you might be infected with hepatitis B or hepatitis C.
- You know, or think that you might be infected with HIV – the AIDS virus
- You have a sexual partner who is infected with hepatitis or HIV
- You are unwell at the moment
- You are anemic or receiving treatment for anemia or iron deficiency
- You are, or may be, pregnant
- You have given blood in the last 1 month (if more than 100 ml is requested)

**Questions before study:**

The only question that will be asked is if the patient is a diabetic?

Are you a hemophiliac?

**What will happen if you take part in this study?**

If you agree to be in this study, you will go to lab 131 and give a blood sample, about 10 drops of blood. You will be seated and blood will be drawn by providing you with a lancet and you will prick your index finger. One small vial of blood will be taken. This will take about five minutes. In addition, we will also be collecting your tears, by providing the participant with Kryolan Tear Stick, a commercially available lachrymator. The Kryolan will be rubbed 15 millimeters under the eye, the warmth from the skin will release menthol vapors, which will rise to the eyes and cause tears to come out. The tears will be collected and measure on the glucometer instrument.

**Are there risks?**

The lancet will hurt. There is a risk of bruising.

**Are there benefits?**

There is no benefit to you. The blood will be used only for laboratory research.

**Can I say “No”?**

Yes, you do not have to donate a blood sample for this study. If you decide not to donate, it will not affect your job standing, class standing, grades, status on an athletic team, or our friendship.

**Taking part is voluntary:** Taking part in this study is completely voluntary. You may skip any questions that you do not want to answer. If you decide not to take part or to skip some of the questions, it will not affect your current or future relationship with the researchers. If you decide to take part, you are free to withdraw at anytime.

**Will my personal/medical information be kept confidential?**

We will do our best to protect the information we collect from you and/or your medical record. Information which identifies you will be kept secure and restricted. However, your personal information may be given out if required by law. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information which identifies you will be destroyed when this research is complete. The following organizations may look at information about you in your medical and research records: Institutional Biosafety committee.

**Are there any costs or payments?**

You will NOT be paid for taking the time to donate a blood sample. You will NOT be charged for the blood sample.

You may be requested to provide your name and date of birth. This information will be disclosed one time to either Bard College for purposes of appreciating you for participation in this study.

**Who can answer my questions about the study?**

You can talk to the study investigator about any questions or concerns you have about this study. Contact the study investigator(s) Ayda Gonzalez at (347) 337-6103.

If you have any questions about your rights as a participant in a research project or for more information on how to proceed should you believe that you have been injured as a result of your participation in this study you should contact the study investigator.

.....

**CONSENT TO PARTICIPATE**

**Statement of consent:** I have read the above information, and have received answers to any questions I asked. I consent to take part in the study. I have been given copies of this consent form to keep.

If you wish to be in this study, please sign below.

\_\_\_\_\_  
Participant's Signature and Today's Date

\_\_\_\_\_  
Participant's Name (Printed)

\_\_\_\_\_  
Participant's Date of Birth

\_\_\_\_\_  
Person Obtaining Consent Signature and Date

\_\_\_\_\_  
Person Obtaining Consent Printed

Name of Principal Investigator: Ayda Gonzalez  
Address: 30 Campus Rd Annandale-On-Hudson, NY, 12504  
Telephone Number: (347) 337-6103

Name of Faculty Sponsor: Christopher LaFratta  
Address: 30 Campus Rd Annandale-On-Hudson, NY, 12504  
Telephone Number: (845) 752-2353

**If you have questions:** The researcher conducting this study is Ayda Gonzalez under the supervision of Dr. Christopher LaFratta. Please ask any question you have now. If you have questions later, you may contact Ayda Gonzalez at [ag0981@bard.edu](mailto:ag0981@bard.edu) or at (347) 337-6103. If you have any questions or concerns regarding this study, you may contact the Institutional Review Board (IRB) at [irb@bard.edu](mailto:irb@bard.edu) or access their website at <http://www.bard.edu/irb/>.

### ***5.3 Questions for participant before the experiment***

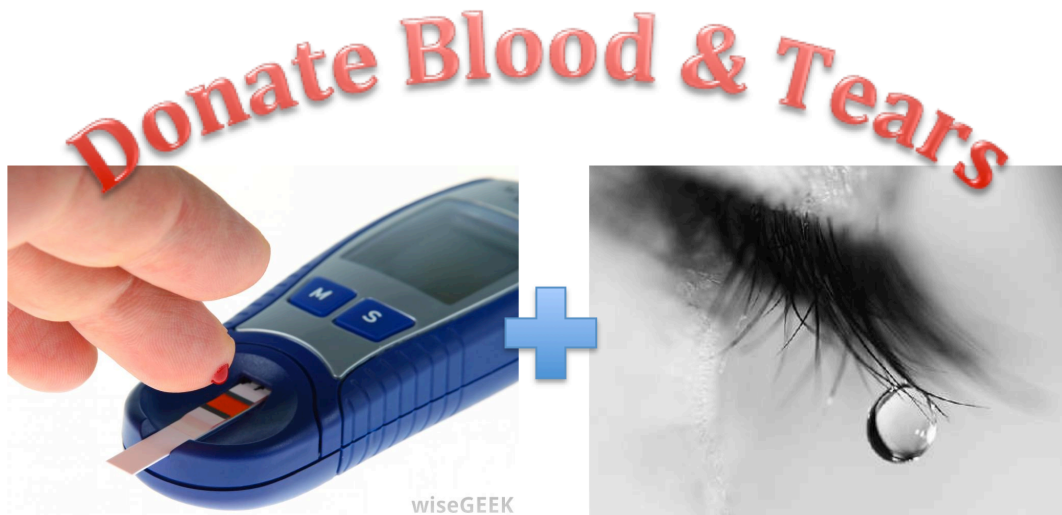
#### **Preliminary Questions:**

The answers to these questions will be confidential. Please read the questions carefully and check yes or no in the appropriate box. These questions will determine if you are eligible to participate in this study.

<b>Full-Length Volunteer History Questionnaire (Answer yes or no)</b>	<b>YES</b>	<b>NO</b>
<b>Are you...</b>		
1. Feeling healthy and well today?		
2. Currently taking an antibiotic?		
3. Currently taking any other medication for an infection?		
4. Have you taken aspirin or anything that has aspirin in it?		
<b>In the past 6 weeks</b>		
5. Female donors: Have you been pregnant or are you pregnant now?		
<b>In the past 8 weeks have you</b>		
6. Donated blood, platelets, or plasma?		
7. Had any vaccinations or other shots?		
8. Had contact with someone who had a smallpox vaccination?		
<b>In the past 12 months have you</b>		
9. Had a blood transfusion?		
10. Come into contact with someone else's blood?		
11. Had an accidental needle-stick?		
12. Had a tattoo?		
<b>Have you EVER...</b>		
13. Had a positive test for the HIV/AIDS virus?		
14. Had a bleeding condition or a blood disease?		

#### 5.4 Poster for advertisement

Ever wondered if there was a correlation between glucose in tear fluid and blood?



A drop of blood, **hope for less pain!**

- Where: RKC 131
- When: February 20-May 1
- Contact: Ayda Gonzalez (347) 337-6103
- How: By appointment, please email [Ag0981@bard.edu](mailto:Ag0981@bard.edu)

**IMPORTANT:** For the purpose of studying the correlation between blood glucose and tear glucose, volunteers can donate a few drops of blood from the capillaries of their fingers that can be safely collected by them pricking themselves with a lancet, which is usually used at home by diabetic patients.

Tears will then be collected from the same participants by blowing enough air to their eyes, in a safe way, until they tear up, in which then the samples will be collected and measure with a glucometer.

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